**Abstract**

Many metabolic and autoimmune conditions are frequently hard to treat and even more difficult to cure. Often times the best outcome is a lifetime of drug treatment. However, new research is showing the microbiome has a significant influence on many diseases, especially in the realm of autoimmune conditions and obesity. There are functional and core genetic differences in the microbiomes of individuals with these conditions. This new area of research is impacting the way medicine treats diseases, as the microbiome is more malleable than the human genome. It can be modified through a variety of environmental exposures. Medically, these modifications include antibiotics and, more recently, fecal microbiota transplants (FMTs). The evidence suggests that modifying the microbiome in order to affect disease outcomes has the potential to provide an important additional approach to treatment.

## Introduction

The gut microbiota constitutes an essential ecosystem of bacteria, fungi and viruses located in the human intestine that is vital to normal health. Studies with germ free mice have documented the important role the gut microbiota plays with respect to maintaining overall health. With more than 1,000 distinct bacterial species identified to-date, we now know that while each person has a unique microbiome, there are normal common 'core' genes among bacterial species that share a similar functional role based on common shared genes rather than common bacterial species. Similarities among bacterial species are seen within geographic regions. Significant shifts in this microbiota system can trigger disease and dysbiosis, a condition with microbial imbalances on or inside the body. The vast majority of bacteria belong to superkingdoms Bacteroidetes and Firmicutes. Shifts in the ratio of these two groups of bacteria can result in dysbiosis, which occurs with obesity and inflammatory bowel disease (IBD). How the microbiome changes is an area of active research. Such changes can trigger an inflammatory response and result in autoimmune conditions. The potential impact on public health and clinical practices could include using microbiome genomics to identify and diagnose abnormalities and diseases as well as optimize the treatment of specific diseases related to gut microbiota dysbiosis.

## Intestinal Permeability and Disease

Molecular analyses have shown that the composition of the human intestinal microbiota is individually specific, with each person having a unique microbiome which is relatively stable over time (1, 2, 3).

An increasing number of diseases have been linked to both alterations in the microbiome and in intestinal permeability due to zonulin protein regulation. Zonuiln works to control the spaces between cells of the instestine, the so-called tight junctions. By opening the tight junctions, zonulin allows some substances to pass, while others, such as bacteria or toxins, are kept out. If zonulin activation is dysregulated, for example by inflammation, then increased intestinal permeability occurs. Diseases related to "leaky" tight junctions include many autoimmune conditions such as including IBD (4, 5, 6), Type 1 Diabetes (7, 8, 9, 10), multiple sclerosis (11), celiac disease (12, 13), rheumatoid arthritis (14), psoriasis (15), eczema (16), and central nervous system disorders such as autism (17).

If dysbiosis that includes foreign microbes occurs in a genetically predisposed individual, then an inflammatory response occurs, which can allow the passage of antigens through the intestinal tight junctions. This stimulates an immune response, which may target certain organs or tissues in these individuals and leads to a triggering of an autoimmune condition. Dysbiosis and the dysregulation associated with tight junctions is also associated with cancer development, infections, and allergies (18-25). Research has shown that the resolution of symptoms for many conditions ranging from autoimmune diseases to obesity can be addressed through manipulation of the microbiome (26, 27).

Fluctuations in gut permeability can also help explain immune responses that are activated through regulation of antigens, such as allergens or foreign microbes, through tight junctions by the zonulin pathway. Zonulin activation causes intestinal epithelium to disassemble tight junctions and leads to increased gut permeability, which causes an immune response that can trigger predisposed autoimmune conditions (28).

Enteric infections have been implicated in the pathogenesis of several conditions, including allergic, autoimmune, and inflammatory diseases, by causing impairment of the intestinal barrier through the activation of zonulin (29). Microorganisms can change the intestinal permeability either directly from an allergen, such as gliadin, or through elaboration of toxins from bacteria, such as C. difficile (30). Increased intestinal permeability precedes disease by causing prolonged zonulin activation and an excessive antigen release. This can trigger certain diseases in the genetically predisposed individual once threshold levels of inflammation are reached. (31-34). The autoimmune effect dysbiosis can have on

the GI tract is seen in IBD, and correction of the abnormal flora can reverse symptoms of IBD (40, 48-49).

## Inflammatory Bowel Disease

Increased intestinal permeability, as well as microbiome dysbiosis, has also been shown to play a central role in the pathogenesis of inflammatory bowel diseases (IBD such as Crohn's disease and ulcerative colitis (UC) (35,36). Zonulin activity and increased intestinal permeability are detectable in the acute phase of IBD. (37, 38).

A primary defect of the intestinal barrier function could be involved in the early steps of the pathogenesis of IBD. The prolonged inflammatory state and production of cytokines maintain the increased permeability, creating a vicious cycle. Barrier dysfunction allows further leakage of intestinal contents, and a continued immune response is made to counteract the leakiness (39).

Dysbiosis and decreased complexity of the gut microbial ecosystem are also common features in patients with Crohn's disease and ulcerative colitis (40). IBD patients have an abnormal composition of gut microbiota and host immune dysregulation. The abnormal components of gut microbiota cause genetically susceptible hosts to create inappropriate innate and adaptive immune responses (41).

Chronic recurrent inflammation associated with ulcerative colitis has been linked to persistent infection. Specifically, *E. coli* and *B. vulgatus* contribute to the dysbiosis and may play a harmful role in the pathogenesis of IBD. Both are normal members of the flora, so further research is needed to understand the mechanisms for their increased presence in IBD (42-45). Better understanding of the microbe functions in these diseases will allow for future treatments to restore the specific abnormalities seen in these conditions.

## Obesity and Metabolic Diseases

Numerous studies have shown that gut microbiome play a role in metabolic disorders such as obesity, diabetes, and cardiovascular diseases. The microbiota affect metabolic diseases by creating an innate immune response to dysbiosis as seen in the biome of obese individuals, and bacterial metabolites that affect host metabolism (such as butyrate or SFCA) (50).

Obesity is characterized by persistent low-grade inflammation and alterations in gut motility. Current research is examining the role of gut microbiota as an environmental factor that contributes to obesity. The gut microbiota can affect obesity and increase inflammatory responses by modifying the intestinal lining.

As with IBD, a vicious cycle of inflammation & increased gut permeability ensue. Inflammatory conditions influence the excitability of enteric neurons and may add to the gut dysfunction in obese individuals (51).

Reduced bacterial diversity, altered representation of necessary core bacterial genes, and the resulting altered metabolic pathways are linked with the microbiome of obese individuals, reinforcing the evidence that abnormalities in the core functional microbiome are associated with different metabolic effects (52).

Dysbiosis can also lead to changes in host gene expression, which can affect gut epithelial cells as well as endocrine functions. Alterations in intestinal microbiota are associated with obesity (53). Differences in gut microbes of obese individuals indicate that the obese microbiome has an increased ability to extract energy from the diet. Studies in mice have shown that introduction of an 'obese microbiome' results in a significantly greater increase in total body fat than colonization with a 'lean microbiome'.

The microbiome's role in digestion has major implications for obesity as well as malnutrition. Studies of obese mice compared their microbiome with that of mice with a normal weight, and significant differences were found. The obesity-associated microbiome had a significantly higher amount of bacterial genes that encode enzymes used to digest dietary polysaccharides (55).

Shared 'core' microbial genes among individuals have been identified at the gene rather than organism level. Obesity is associated with phylum-level changes in the microbiota, specifically lower Bacteroidetes and higher Firicutes ratios, which showed up to 150 kcal increase in energy extraction from food with a 20% increase in Firicutes bacteria . Overfeeding of lean mice also led to microbiota changes in the gut, indicating that diet affects microbiome (56-62).

Another recent study showed Bacteroides presence along with a healthy diet in obese mice caused weight loss. This finding showed specific members of Bacteroidetes from the microbiota of lean mice and a low saturated fat, high fruit/vegetable diet led to a rapid, modifiable weight loss from diet and microbiota interactions (70).

## Malnutrition

Kwashiorkor, a form of malnutrition due to protein deficiency, could be a type of malnutrition that will not be cured with temporary dietary interventions alone. A study in Malawi analyzed the microbiome differences among healthy and malnourished groups. When gut bacteria in Malawian children with Kwashiorkor were compared to Malawian children with healthy BMIs a significantly less diverse biome was observed in the sick children.

FMTs were performed in mice to study the effects of re-feeding both groups a nutrient-dense diet, and the mice with Kwashiorkor biome could not gain weight as effectively. Ready to use therapeutic foods didn't change the microbiome of the Kwashiorkor group. Once traditional Malawian diet resumed, symptoms returned. These findings suggest the gut microbiome is a contributor to Kwashiorkor.

Additionally, abnormal sulfur metabolism could be due to biome differences. The Malawian diet contains very little cysteine or methionine, so is naturally low in sulfur. A bacterium called *Biophilia wadsworthia* consumes a significant amount of sulfur from the diet. This bacteria is more common in the children with Kwashiorkor, thus it could be exacerbating the effects of an already-deficient diet.

Findings also show that the kwashiorkor-associated microbes can interfere with the tricaroxylic acid cycle and its intermediates, thus making it more difficult for children to harvest energy from their diet, which is already scant of calories.

## Current State of Science

These findings should inform how we approach treatment. While diet plays an important role, the emerging evidence suggests that changing the microbiome may be just as important. Research is still needed to understand the functional roles of bacterial genes. Future treatment could involve tailoring the diet to individual microbiomes. Eventually, technology could advance so that health professionals can analyze gut microbes and tailor the diet based on the bacterial content (64). While researchers continue to probe the microbiome, treatments for specific diseases, such as for infection with *Clostriudium difficile*, have been treated with fecal microbiota transplants. These transplants are used to restore a healthy microbiota.

## The State of FMTs

FMTs are exactly as they sound. After a laxation preparation is administered, a transplant of feces is infused through an nasogastric or nasoduodenal tube, or rectally via an enema or colonoscopy. Freshly produced donor stool (200–300 g dissolved in 500 mL of saline) is administered, and the patient remains horizontal for as many hours as possible to maximize effectiveness. Effectiveness is similar among the different routes of administration for treating UC and *C. Diff.* The host microbiome changes will vary depending on disease state and severity (single or multiple FMTs) (65). The effect of fecal transplants is thought to re-establish healthy microbiome, but what exactly happens and how remains unclear. The process is typically repeated daily for anywhere from 5 days to a few months,

depending on the patient's condition, and may or may not be preceded by an antibiotic regimen (67). Current research to support FMTs in conditions besides *C. Difficile* is poor, with the bulk of evidence being individual case studies and studies in mice. The existing research studies suggest promise for future FMT research in humans.

As of this date, the FDA has classified FMTs as an Investigational New Drug due to the lack of randomized controlled trials as well as unresolved issues regarding informed consent, donor screening, liability, privacy, and ethics in attempts to make the process safer. In addition, the fecal screening process is a costly and time consuming. For example, the current screening process of donors for fecal transplantation includes:

1. No diarrhoea or irritable bowel complaints
2. Normal BMI (18–25 kg/m2)
3. No family history of autoimmune diseases (type 1 diabetes, Hashimoto hypothyroidism, Graves hyperthyroidism, rheumatoid arthritis, inflammatory bowel diseases eg Crohn's disease, Colitus ulcerosa or coeliakie)
4. No HIV, HAV, HBV, HCV, active CMV, active EBV (donor and acceptor are matched for EBV/CMV immune status)
5. No unsafe sex practice or use of illicit drugs
6. Screening of fecal bacterial pathogens (salmonella, Shigella, Campylobacter, Yersinia, Helicobacter pylori antigen), viruses (rotavirus) or parasites (ova and parasites, Giardia antigen, cryptosporidium antigen)
7. Negative C. difficile stool test and/or current communicable (intestinal) disease
8. Any medication use including PPI and antibiotics in the last 3 months
9. No traveling to areas with endemic diarrhea in the last 3 months
10. No immunosuppressive or chemotherapeutic agents (65).

The time-consuming protocols, 30-day waiting periods and of the paperwork involved has led to the development of an underground implementation process. Instead of going through traditional medical procedures, some people are performing the relatively simple FMT procedure at home so as to avoid the bureaucratic burden (66).

The actual FMT process is so simple that some doctors give do-it-yourself instructions for patients to perform the process at home. There are also numerous websites as well as YouTube instruction videos for the procedure (66).

## Implications for Public Health Nutrition Interventions

As discussed above, abnormalities in the core functional microbiome are associated with different metabolic and inflammatory effects, which could be used as a diagnostic tool for treatment potential by modifiying the microbiome for diseases like IBD and obesity based on strains of bacteria that are found in abnormal ratios (63).

Future research is needed to identify candidate microbial biomarkers that may eventually be used in diagnosis and individualized treatment of disease. For example, some microorganisms such as bifidobacteria and lactobacilli are considered to have protective effect against inflammatory bowel disease (68).

Methods could be used to determine and extract the microbiota that help people absorb the maximum possible value from food. This could be a future prophylactic measure in preventing malnutrition (69).

Isolating and unveiling the properties and functions of a healthy microbiota could provide targets for nutrition interventions by modifying microbes in order to maintain wellness in healthy populations and reestablish symbiosis in those who have disrupted microbiota and their associated diseases. Identifying specific bacterial strains or genotypes to diagnose and treat disease is also an important approach to consider for future therapeutic developments to address various GI, autoimmune, and metabolic diseases.

Understanding the impact of interactions between the gut microbiome and the host metabolism and immune functions is important in maximizing treatment therapies that can improve human health through disease prevention and treatment.

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